Focal Expression of Insulin-like Growth Factor I in Rat Kidney Collecting Duct

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Abstract. To address the question of insulin-like growth factor (IGF) I localization and synthesis in kidney, we used two complementary experimental approaches: immunohistochemistry of fixed paraffin-embedded rat kidney sections; and measurement of IGF I mRNA in isolated components of the rat nephron, using a highly sensitive and specific solution hybridization assay. Immunostainable IGF I was localized exclusively to principal cells of cortical and medullary collecting ducts. Administration of growth hormone to hypophysectomized rats for 8 d resulted in enhanced immunohistochemical staining of IGF I within collecting ducts, but no detectable IGF I in other portions of the nephron. The abundance of IGF I mRNA was 7–12-fold higher in isolated papillary collecting ducts than in proximal tubules or glomeruli, and was enriched 10-fold compared with whole kidney. Our data demonstrate colocalization of IGF I and IGF I mRNA in the collecting duct, consistent with focal expression of the IGF I gene at this site.

INSULIN-like growth factors (IGFs)[1] I and II are peptides with ~50% structural identity with insulin. The "somatomedin" hypothesis holds that somatotrophic actions of growth hormone are mediated through growth hormone–stimulated production and/or release of one or both of these peptides, and subsequent interaction of IGFs with receptors on sensitive cells (6, 11). The liver is thought to be the major site of synthesis for circulating IGF I (11). However, evidence has accumulated to suggest that this peptide is produced in a variety of tissues and that locally synthesized IGF I may exert actions on the cells of origin or adjacent cells (6, 7). Evidence for production of IGF I by kidney has come from several sources. D'Ercole et al. (7) reported that administration of ovine growth hormone to hypophysectomized rats increased the amount of IGF I extractable from whole kidney. Others have shown that administration of growth hormone to hypophysectomized rats results in increased levels of IGF I mRNA in kidney, strongly suggestive of local growth hormone–enhanced production (23, 26).

The cell types synthesizing IGF I within kidney are not known with certainty. Studies designed to address this question have used adult and fetal tissues from different species. Andersson et al. (1) localized IGF I to rat proximal tubule using immunohistochemical techniques, consistent with production at this site. Han et al. (19) detected immunostainable IGF I in the lining columnar epithelium of the proximal and distal convoluted tubules and weak staining in collecting ducts of human fetal kidney. Using in situ hybridization, this group found IGF I mRNA in the capsule, calyces, interstitium of inner cortex, and medulla, but not in the nephrogenic zone that includes glomeruli and tubules. Because of the lack of concordance of IGF I mRNA with immunostainable IGF I, they concluded that the immunostained cells do not synthesize IGF I but rather that positive immunostaining in human fetal kidney reflects sequestered peptide (20).

The present studies were undertaken to readdress the questions of IGF I localization and synthesis in adult rat kidney. To this end we used two complementary approaches: (*a*) immunohistochemistry using antiserum directed against IGF I (IGF I antiserum); and (*b*) a solution hybridization assay to detect IGF I mRNA in total RNA extracted from isolated glomeruli, proximal tubules, and papillary collecting duct cells. Our data demonstrate colocalization of IGF I and IGF I mRNA in the collecting duct, consistent with IGF I synthesis at this site.

Materials and Methods

Performance of Nephrectomy, Preparation of Renal Tissue, and Immunohistochemical Staining

Female Wistar or male Sprague Dawley rats aged 12 wk (Harlan Sprague Dawley, Inc., Indianapolis, IN) were anesthetized using intraperitoneal pentobarbital. Kidneys were exposed through a midline peritoneal incision, and perfused with Krebs-Ringers-Phosphate buffer, pH 7.4 (125 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Na_2HPO_4-HCl), to minimize the volume of blood contained in the organ. Perfusion was

1. *Abbreviations used in this paper*: IGF, insulin-like growth factor; pGH, porcine growth hormone; rGH, rat growth hormone; rPRL, rat prolactin.

achieved by catheterizing the aorta distal to the renal arteries, occluding the aorta proximal to the kidneys, transecting the inferior vena cava, and rapidly infusing 100 ml of the buffer, warmed to 37°C, through the distal aortic catheter until complete blanching of the kidneys was apparent. The kidneys were then removed, stripped of their capsules, weighed, cut longitudinally into two halves, and placed in 4% formalin, 2% calcium acetate, pH 7.4, overnight for fixation. Fixed kidneys were embedded in paraffin (22), sliced into 5-μm sections, and placed on gelatin-coated slides in preparation for immunohistochemical staining. Staining was done using an avidin-biotin immunoperoxidase technique (10). Initially, paraffin-embedded tissue sections were deparaffinized by immersion in xylene followed by isopropyl alcohol. After rehydration of the tissue sections with distilled water, slides were immersed in 80% methanol containing 0.6% hydrogen peroxide (15 min) to block endogenous peroxidase activity. Sections were then treated with 1 mg/ml of goat gamma-globulin (Worthington Biochemical Corp., Freehold, NJ) for 30 min to reduce nonspecific binding of biotinylated goat anti-rabbit antibody (see below). Sections were incubated with an avidin-containing solution followed by a biotin wash (Vector Laboratories, Inc., Burlingame, CA) for 30 min each to inactivate endogenous biotin activity. After these treatments, tissue sections were incubated for 2 h with antisomatomedin-C/IGF I rabbit antiserum (kindly provided through the National Institute of Diabetes, Digestive and Kidney Diseases [NIDDK] National Hormone and Pituitary Program by Drs. Louis E. Underwood and Judson J. Van Wyk, University of North Carolina, Chapel Hill, NC). The antiserum was diluted in a PBS consisting of 120 mM NaCl, 40 mM K_2HPO_4, 10 mM NaH_2PO_4, pH 7.4, 5×10^{-5} M thimerosal, and 3% BSA. Alternatively, sections were incubated in identical dilutions of nonimmune rabbit serum (Vector Laboratories, Inc.). Thereafter, a biotinylated goat anti-rabbit antibody (Vector Laboratories, Inc.) was applied for 30 min followed by a horseradish peroxidase-avidin complex (Zymed Laboratories, South San Francisco, CA) for 5 min. Then, aminoethyl carbozol (Zymed Laboratories), in 3% H_2O_2 was applied to the sections for 5 min. Sections were counterstained with hematoxylin (Zymed Laboratories) for 3 min, slides were dried, and coverslips applied. Each of the steps described above was punctuated by immersion of the tissue sections in PBS for 5 min.

Preparation of Isolated Glomeruli, Proximal Tubular Segments, and Segments of Papillary Collecting Duct

Sprague-Dawley rats were anesthetized and kidneys were perfused in the manner described above. Glomeruli were isolated using a sieving technique (21). First, the renal cortex was separated from the medulla and minced with a steel blade. The minced cortex was sequentially forced through the mesh of 250-μm and 150-μm stainless steel filters. The filtrate was then placed on top of the mesh of a 75-μm filter and nonglomerular elements washed through using Krebs-Ringers-Phosphate buffer, pH 7.4. Glomeruli were retained atop the mesh. They were suspended in Krebs-Ringers-Phosphate buffer and concentrated at 175 *g* for 1 min using a table top centrifuge. This procedure results in the isolation of ~3 mg of glomeruli per kidney of a 150-g rat. Greater than 90% of cells within the glomeruli exclude trypan blue consistent with good viability.

Proximal tubular segments were prepared using a Percoll gradient-centrifugation technique exactly as we have described for canine kidney (18). This procedure results in isolation of ~20 mg of proximal tubular segments per kidney from one 150-g rat. Greater than 90% of cells within proximal tubular segments exclude trypan blue, consistent with good viability. Plasma membranes were prepared from cells within proximal tubular segments, as we previously described, and used for measurement of parathyroid hormone and arginine vasopressin-sensitive adenylate cyclase activities (3, 18).

Segments of papillary collecting duct were isolated using methodology described by Stokes et al. (32). Renal medulla, extending from the papillary tip to the margin dividing the outer medulla from the cortex, was removed from the kidneys and placed in ice-cold modified Ringers solution consisting of 118 mM NaCl, 16 mM Hepes, 17 mM Na-Hepes, 14 mM glucose, 3.2 mM KCl, 2.5 mM $CaCl_2$, 1.8 mM KH_2PO_4, pH 7.4. The tissue was minced, gassed with room air, and incubated at 37°C in the same buffer containing 0.2% collagenase (Cooper Biomedical, Frankfort, FRG) and 0.2% hyaluronidase (Boehringer Mannheim GmbH, Mannheim, FRG). Tissue from 12 papillae was suspended in 10 ml of buffer. After 45 min, 0.001% DNase (Boehringer Mannheim GmbH) was added to prevent clumping. Suspended tissue was aspirated through a Pasteur pipette 10–12 times every 15 min until there was no visible clumping (usually 45 min additional). The suspension was then centrifuged at 175 *g* for 2 min, the supernatant discarded, and the pellet resuspended in buffer without DNase. Centrifugation and resuspension were repeated twice. Segments contained in the resulting pellet were used in experiments. This technique results in isolation of ~16 mg of papillary collecting duct segments per one kidney of a 150-g rat. Greater than 90% of cells within papillary collecting duct segments exclude trypan blue, consistent with good viability. Plasma membranes were prepared from cells within papillary collecting ducts using methodology described by Gluck and Al-Awqati (13). Segments were suspended in 250 mM sucrose, 5 mM Tris-HCl, 1 mM $NaHCO_3$, 1 mM dithiothreitol, 1 mM EGTA, pH 8.0, and homogenized using a Teflon glass homogenizer. The homogenate was centrifuged at 6,000 *g* for 15 min and the resulting supernatant centrifuged at 38,000 *g* for 1 h. The fluffy upper layer of the pellet was retained and used for measurements of arginine vasopressin-sensitive adenylate cyclase activity.

Isolated glomeruli, proximal tubular segments, and segments of papillary collecting duct were fixed as described above for whole kidneys and 5-μm sections placed on gelatin-coated slides in preparation for staining with hematoxylin and eosin.

Administration of Growth Hormone to Hypophysectomized Rats, Performance of Nephrectomies, and Preparation of Renal Tissue

16 hypophysectomized Wistar rats (130 g) (Harlan Sprague Dawley, Inc.) were fed ad libitum. Four rats were injected with rat growth hormone (rGH), obtained from the National Hormone and Pituitary Program of NIDDK (75–100 μg per day subcutaneously), four were injected with the same amount of recombinant porcine growth hormone (pGH, obtained from American Cyanamid, Princeton, NJ), four were injected with the same amount of rat prolactin (rPRL) obtained from NIDDK, and four were injected with vehicle. Rats were weighed between 3:30 and 5:00 p.m. every afternoon. After 8 d of injection, rats were anesthetized with pentobarbital, and kidneys were removed and weighed. Two left kidneys from each group of rats were used for immunohistochemical staining. These kidneys were perfused, fixed, and sliced as described above. The remaining kidneys were snap-frozen in liquid nitrogen in preparation for extraction of RNA (see below). Before they were killed, sera were obtained from rats for measurement of levels of circulating IGF I.

Extraction of RNA from Renal Tissue

RNA was extracted from whole kidney by homogenizing tissue in 10–15 vol of 4 M guanidinium thiocyanate, 50 mM Tris-HCl, 10 mM EDTA, 2% sarkosyl, 1% beta-mercaptoethanol, pH 7.0, as described by Chirgwin et al. (4). The integrity of each sample was verified by electrophoresis on an agarose/formaldehyde gel (26), and its quantity was determined spectrophotometrically.

RNA was extracted from glomeruli, proximal tubular segments, and segments of papillary collecting duct by homogenization in 5 vol of guanidinium thiocyanate solution followed by centrifugation through a gradient of CsCl (4). The integrity and quantity of each sample were determined as described above.

RNA Analysis

A solution hybridization nuclease protection assay for IGF I mRNA was performed as described previously (30). ^{32}P-labeled single-stranded probes derived from a subclone of rat IGF I exon 3 in pGEM2 (29) (Promega Biotec, Madison, WI) were synthesized in an "antisense" orientation using T7 RNA polymerase (Bethesda Research Laboratories, Gaithersburg, MD), [α-^{32}P]CTP (800 Ci/mmol, New England Nuclear, Boston, MA), and unlabeled ATP, GTP, and UTP, according to the protocol of Zinn et al. (35). A subclone consisting of most of the first protein-coding exon of rat IGF II and adjacent intron sequences was prepared in pBS (Strategene Cloning Systems, San Diego, CA) and transcribed in the "antisense" orientation using T3 polymerase as described above (27). RNA samples were hybridized with labeled probes, hybrids were digested sequentially with RNases A and T1 (Sigma Chemical Co.) and proteinase K (Bethesda Research Laboratories), and RNA was extracted, precipitated, and washed as before (27, 30). Samples were dissolved in sequencing dye, heated for 3 min at 90°C, cooled, and applied to a 6% acrylamide/8.3 M urea DNA sequencing gel. Under these conditions, the protected IGF I band migrates as a single fragment of 182 nucleotides and the protected IGF II fragment appears as a doublet of 151–152 bases (27, 28). After electrophoresis, the gel was dried in vacuo and exposed to Kodak XAR film at −80°C for 5–24 h using a light-

ning plus intensifying screen (DuPont Co., Wilmington, DE). In all experiments yeast tRNA was included as a negative control and liver RNA as a positive control. All experiments were performed in triplicate. Relative abundance of IGF I and IGF II mRNA was calculated using a scanning laser densitometer (LKB Instruments Inc., Bromma, Sweden).

Radioimmunoassays

Radioimmunoassay for IGF I in rat plasma was performed as previously described (5). Hormone-sensitive adenylate cyclase activity in plasma membranes prepared from cells within proximal tubular segments and papillary collecting ducts was measured using a radioimmunoassay for cAMP (3).

Results

To immunostain for IGF I, we incubated fixed paraffin-embedded sections of kidney originating from female Wistar or male Sprague-Dawley rats with different concentrations of IGF I antiserum (1:200, 1:250, 1:500, 1:1,000, or 1:1,250 dilutions). As a control we substituted identical dilutions of nonimmune rabbit serum. Incubation with IGF I antiserum resulted in specific staining of cortical and medullary collecting ducts in kidneys from both strains and sexes of rat. Staining was not observed in glomeruli, proximal tubules, loops of Henle, or distal tubules. Intensity of coloration was highest with 1:200–500 dilutions of antiserum. Fig. 1 contains representative photomicrographs of kidneys from Sprague-Dawley rats incubated with a 1:200 dilution of IGF I antiserum. These photomicrographs demonstrate localization of IGF I within cortical (Fig. 1 *b*) and medullary (Fig. 1 *d*) collecting ducts. IGF I is found within all cells of medullary collecting ducts. However, in cortical collecting ducts there are unstained cells (Fig. 1 *b*, *arrowheads*). These cells correspond to so-called "dark cells" (intercalated cells) (34) demonstrable in hematoxylin and eosin-stained sections sliced adjacent to immunostained sections (Fig. 1, *e* and *f*). Thus, only the "principal" cells (34) of cortical collecting duct stain for IGF I.

To determine whether immunohistochemical localization of IGF I is affected by growth hormone, we administered rGH, pGH, rPRL, or vehicle to hypophysectomized female Wistar rats for 8 d and obtained kidneys from each group of rats at the time they were killed for immunostaining. As expected (11, 23, 26, 33), administration of growth hormone resulted in several anabolic responses, including increases in body weight and kidney weight, elevations in serum IGF I levels (Table I), and specific increases in IGF I mRNA in whole kidney (Fig. 2).

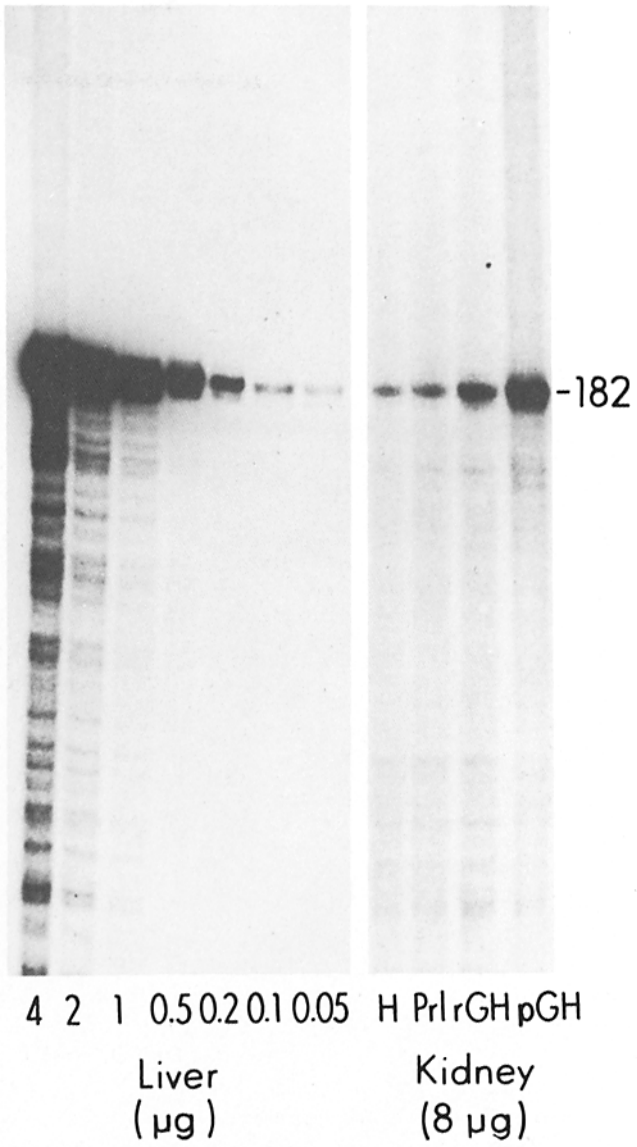


Figure 2. Autoradiogram depicting the results of a solution hybridization nuclease protection experiment, using a ^{32}P-labeled rat IGF I exon 3 antisense probe (protected fragment = 182 nucleotides). Illustrated are protected RNA fragments from rat liver and from whole kidneys originating from hypophysectomized Wistar rats injected with vehicle (*H*), rPRL, rGH, or pGH.

Table I. Results of Growth Hormone or Prolactin Administration to Hypophysectomized Rats

Parameter (units)	Administered agent			
	Vehicle	rPRL	rGH	pGH
Body weight (g) (n = 4)	134 ± 3.3	143 ± 6.3§	161 ± 4.8*	162 ± 4.7‡
Kidney weight (g) (n = 8)	0.45 ± 0.03	0.48 ± 0.03§	0.61 ± 0.03‡	0.58 ± 0.04*
IGF I (ng/ml) (n = 4)	<0.1	181 ± 84	1,039 ± 170	1,022 ± 129

Values are mean ± SEM.
* Greater than vehicle, $p < 0.05$ (Dunnett's multiple comparison procedure; reference 8).
‡ Greater than vehicle, $p < 0.01$.
§ Greater than vehicle, p = NS.

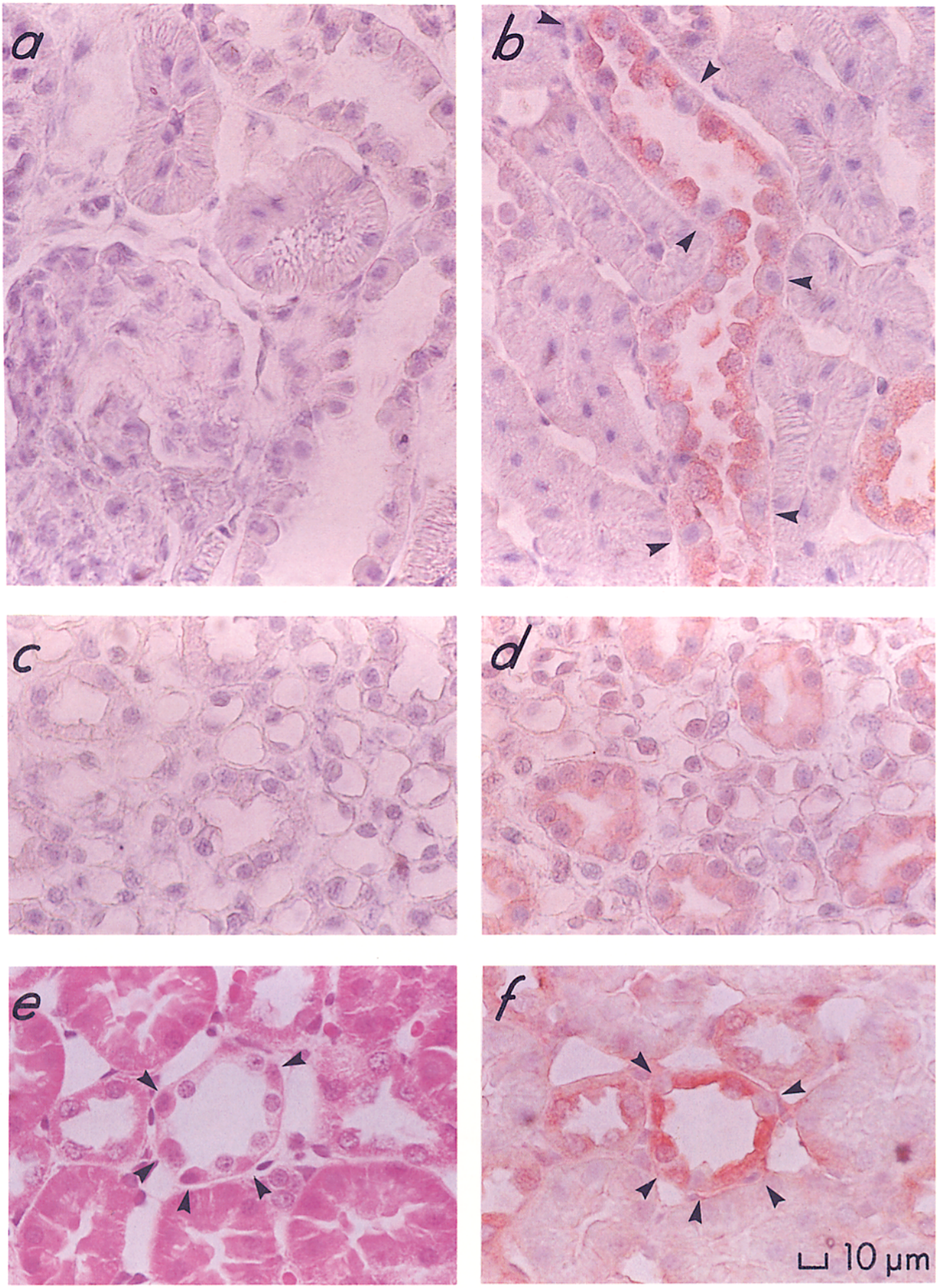


Figure 1. Immunohistochemical localization of IGF I in rat kidney: (*a*) Sprague-Dawley rat cortex, negative control; (*b*) Sprague-Dawley rat cortex, IGF I antiserum; (*c*) Sprague-Dawley rat medulla, negative control; (*d*) Sprague-Dawley rat medulla, IGF I antiserum; (*e*) Sprague-Dawley rat cortical collecting duct stained with hematoxylin and eosin; and (*f*) Sprague-Dawley rat cortical collecting duct in a section immediately adjacent to the section used in *e*, corresponding to the duct illustrated in *e* immunostained using IGF I antiserum. Arrowheads point to some of the nonstaining cells and "dark" cells.

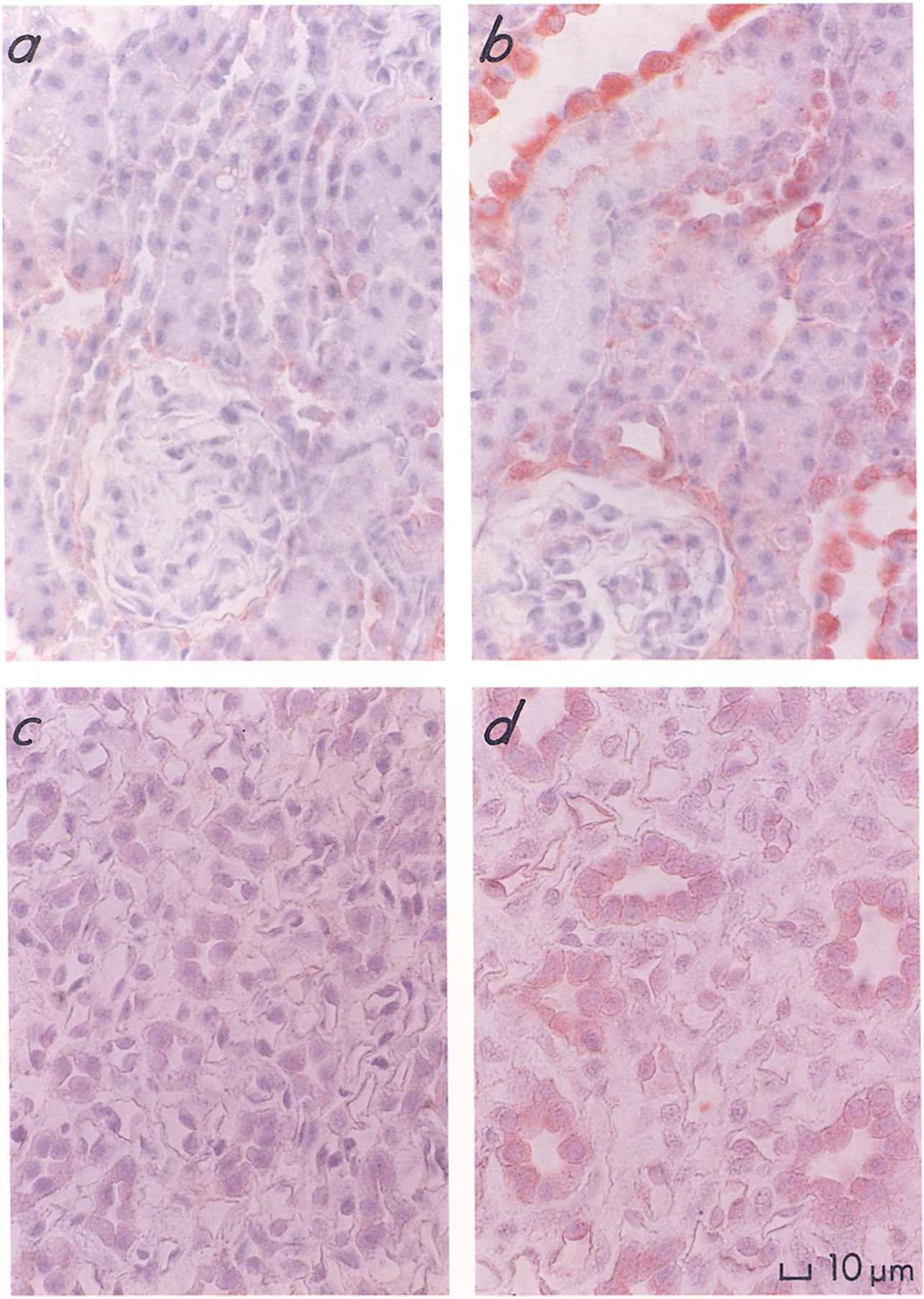
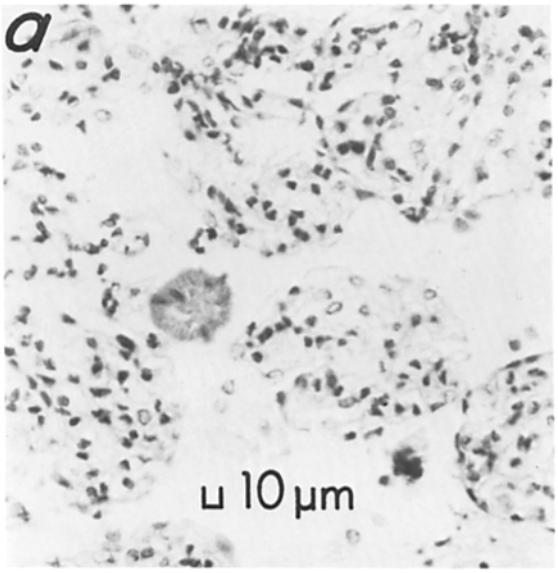
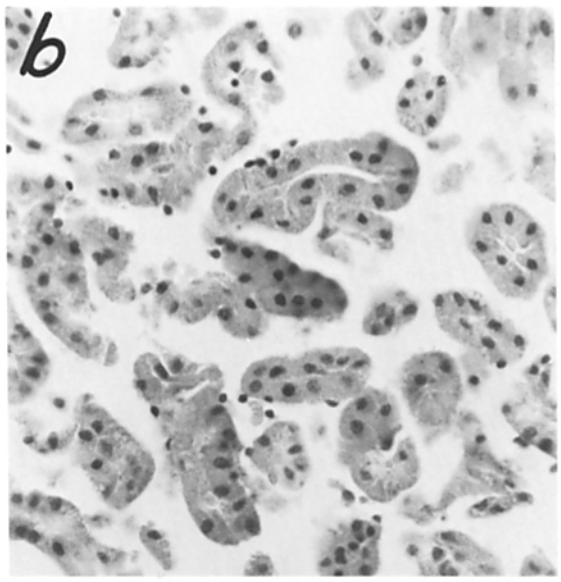


Figure 3. Immunohistochemical localization of IGF I in rat kidney: (*a*) hypophysectomized Wistar rat kidney cortex; (*b*) hypophysectomized Wistar rat kidney cortex obtained after 8 d of injection of pGH; (*c*) hypophysectomized Wistar rat kidney medulla; and (*d*) hypophysectomized Wistar rat kidney medulla obtained after 8 d of injection of pGH. A 1:500 (*a* and *b*) or 1:250 (*c* and *d*) dilution of IGF I antiserum was used in these experiments.





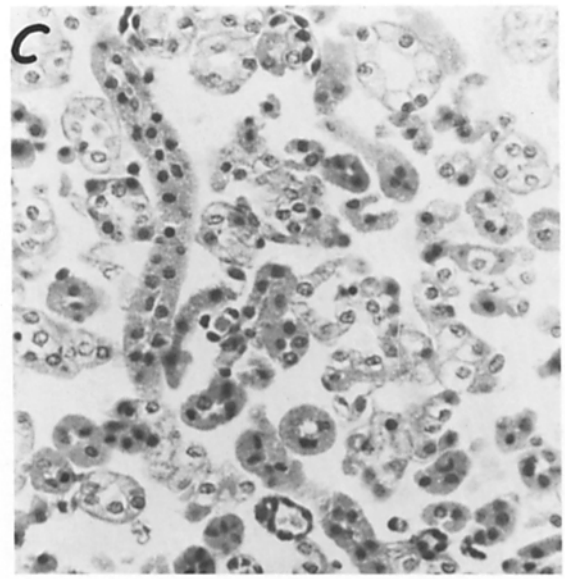


Table II. Adenylate Cyclase Activities in Membranes from Isolated Cells

Conditions	Adenylate cyclase activity (pmol cAMP/mg protein^{-1} per 30 min^{-1})	
	Membranes from proximal tubular segments	Membranes from papillary collecting duct
Basal	208 ± 24	228 ± 62
PTH (10^{-7} M)	795 ± 58*	- - - -
AVP (10^{-7} M)	213 ± 24	506 ± 108‡
NaF (10^{-2} M)	1,954 ± 70	2,279 ± 754

Values are means ± SEM; $n = 4$ (proximal tubular segments) or $n = 5$ (papillary collecting duct) preparations. Adenylate cyclase activities were measured in membranes incubated in the absence of added hormone (*Basal*), or incubated in the presence of parathyroid hormone (*PTH*), arginine vasopressin (*AVP*), or NaF.
* PTH > basal, $p < 0.01$ (Dunnett's multiple comparison procedure).
‡ AVP > basal, $p < 0.05$.

Administration of growth hormone to hypophysectomized rats also resulted in enhanced intensity of immunostainable IGF I in both cortical (Fig. 3, *a* and *b*) and medullary (Fig. 3, *c* and *d*) collecting ducts. Administration of rPRL to hypophysectomized rats did not increase immunostaining for IGF I (not shown). These data indicate that levels of IGF I within cells of renal collecting duct are regulated by growth hormone, consistent with the effect of growth hormone on the abundance of IGF I mRNA within whole kidney.

To determine whether the focal immunohistochemical localization of IGF I within collecting ducts reflects specific local production, we sought to define the distribution of IGF I mRNA within kidney. To this end, we isolated glomeruli, proximal tubular segments, and segments of papillary collecting duct for measurement of IGF I mRNA. Photomicrographs of fixed paraffin-embedded sections of each tissue preparation, stained with hematoxylin and eosin are shown in Fig. 4. This figure demonstrates that our isolation procedures yield preparations of cells with morphologies characteristic of each cell type. Membranes prepared from proximal tubular segments contain parathyroid hormone–sensitive adenylate cyclase activity, but no significant vasopressin-sensitive adenylate cyclase activity. This is consistent with a proximal tubular origin and little contamination with elements of distal nephron (18; Table II). Similarly, membranes prepared from papillary collecting duct segments contain vasopressin-sensitive adenylate cyclase activity, consistent with collecting duct origin (18; Table II).

As shown in Fig. 5, the relative abundance of IGF mRNA in isolated glomeruli, proximal tubular segments, and segments of papillary collecting duct matches the distribution of IGF I, as determined by immunohistochemistry, in the intact kidney (Figs. 1 and 3). As measured by densitometric scanning of autoradiograms from three separate experiments, IGF I mRNA is enriched 10-fold in isolated collecting ducts compared with whole kidney, is 12-fold higher than the amount in isolated glomeruli, and 7-fold higher than in isolated proximal tubules. The level of IGF I mRNA in collect-

Figure 4. Photomicrograph of glomeruli (*a*), proximal tubular segments (*b*), and segments of papillary collecting duct (*c*) isolated from kidneys of Sprague-Dawley rats. Preparations were stained with hematoxylin and eosin.

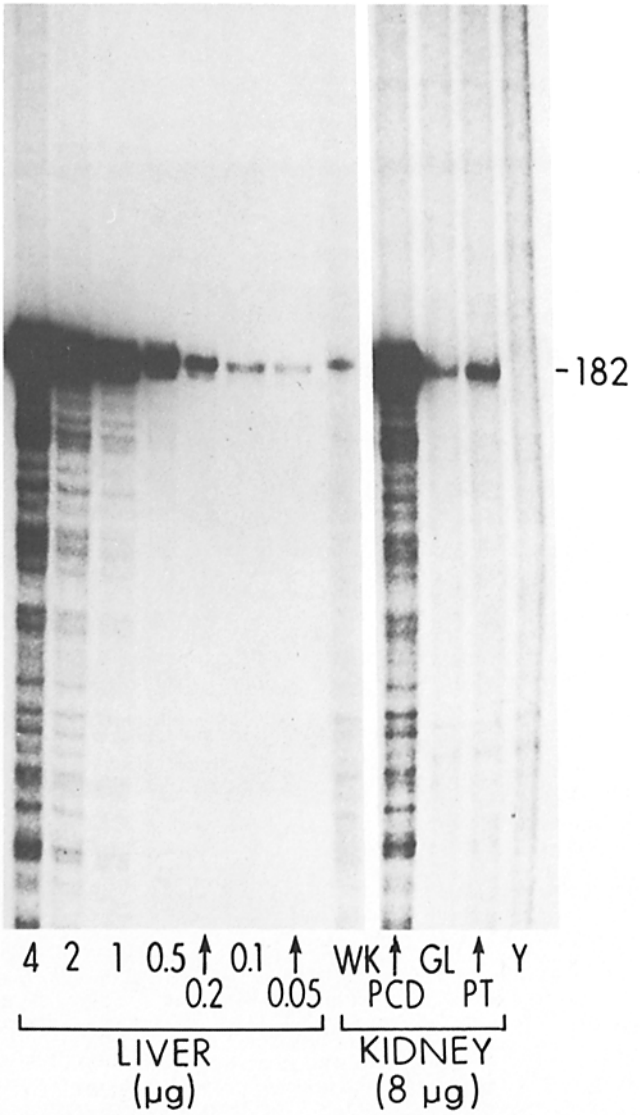


Figure 5. Autogradiogram depicting the results of a solution hybridization nuclease protection experiment performed as in Fig. 2. Illustrated are protected RNA fragments originating from rat liver, whole kidney (*WK*), papillary collecting ducts (*PCD*), glomeruli (*GL*), proximal tubular segments (*PT*), or yeast tRNA (*Y*).

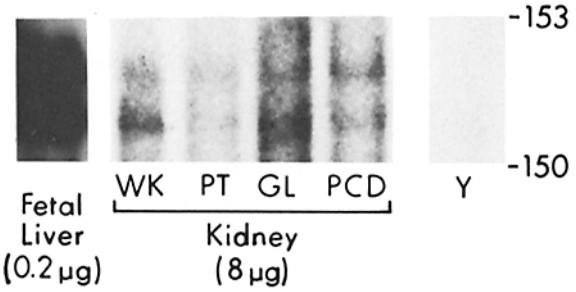


Figure 6. Autoradiograms depicting the results of a solution hybridization nuclease protection experiment using a ^{32}P-labeled IGF II probe. Illustrated are protected RNA fragments from fetal rat liver, whole kidney (*WK*), proximal tubular segments (*PT*), glomeruli (*GL*), papillary collecting ducts (*PCD*), or yeast tRNA (*Y*). Size markers at right are in base pairs.

ing duct is ~25% of that found in liver. To ascertain whether the unequal distribution of IGF I mRNA within different renal cell types reflects the distribution of other mRNAs, we determined the relative abundance of IGF II mRNA in whole kidney, isolated collecting duct, glomeruli, and proximal tubules. As shown in Fig. 6, levels of IGF II mRNA are comparable in whole kidney and in each of the isolated renal cell fractions. This indicates that the focal expression of IGF I in collecting duct is specific.

Discussion

Although data originating in a number of laboratories suggest that IGF I is present in kidney (1, 7, 19, 20, 23, 26), the precise localization of this peptide within renal tissue has not been established with certainty. The findings illustrated in Figs. 1 and 3 of this study indicate that IGF I is present predominantly in collecting duct. In addition, levels of IGF I within collecting duct appear to be regulated by growth hormone. Since the abundance of IGF I mRNA (Fig. 5) parallels the distribution of immunostainable peptide, it appears that the IGF I gene is expressed focally within rat kidney, with a >7:1 gradient favoring cells of collecting duct over glomeruli or proximal tubule. Within collecting duct, there apears to be nonuniform synthesis of IGF I since immunostainable IGF I is present in principal cells, but not intercalated cells of cortical collecting duct.

Ours is the second demonstration of focal synthesis of a polypeptide growth factor within kidney. Rall et al. have previously shown that expression of the prepro-epidermal growth factor gene is localized to the distal tubule of the rat nephron (25). An asymmetrical focal pattern of synthesis of IGF I mRNA has been found in rat central nervous system, where it is concentrated within the olfactory bulb and cervical-thoracic spinal cord (27). Thus, focal expression of IGF I and other growth factor genes within mammalian tissues may be typical.

It is believed that the liver is the major site for IGF I synthesis in rat and that hepatic production of this peptide is sufficient to account for baseline levels of circulating IGF I (29). However, observations from a number of laboratories are consistent with IGF I synthesis also occurring in a variety of nonhepatic tissues including kidney (7, 19, 23, 26). It has been suggested that IGF I produced within kidney may exert actions on the cell of origin (autocrine effects) or on adjacent cells (paracrine effects). Stiles et al. (31) reported increased concentrations of IGF I extractable from kidneys undergoing compensatory hypertrophy after unilateral nephrectomy of rats, compared with kidneys originating in sham-operated control animals. Fagin and Melmed (9) found a relative increase in IGF I mRNA in hypertrophied kidneys of uninephrectomized rats compared to kidneys of sham-operated controls. These observations suggest that locally produced IGF I may be involved in compensatory renal hypertrophy.

It is known that hypertrophy of glomeruli and tubular cells accompanies the elevated levels of circulating IGF I that occur in acromegaly in humans (12). We have characterized specific receptors for IGF I in the basolateral membrane of canine renal proximal tubular cells (17). It is plausible that

proximal tubular hypertrophy is effected, at least in part, through interaction of IGF I produced in kidney with these receptors.

It is of interest to consider the focal expression of IGF I within collecting duct cells of rat kidney in light of the embryological origin of mammalian kidney. The metanephros, from which mammalian kidney develops, has a dual origin. It arises in part from the mesonephric duct and in part from the so-called intermediate mesoderm, a mesodermal ridge located along the posterior wall of the abdominal cavity. The metanephric diverticulum, or ureteric bud, developing from the mesonephric duct, is the first component to appear. As it grows caudally, the metanephric diverticulum branches, gathers about its distal ends intermediate mesoderm, and induces the formation of glomeruli, proximal tubules, loop of Henle, and distal tubules within the intermediate mesoderm. If the metanephric diverticulum is not formed, the metanephric blastema does not differentiate into kidney (16, 24). The metanephric duct and its branches give rise to collecting ducts (16). Thus, the primordia of collecting ducts induce renal organogenesis.

Much has been learned about renal organogenesis by studies carried out in vitro using isolated metanephrogenic tissue. Work by Grobstein (14) and Grobstein and Dalton (15) suggested that induction of renal tubules in intermediate mesoderm isolated from fetal mice is effected by a humoral agent. Whether IGF I is involved in renal tubule formation is unknown and our present studies do not shed light on this issue. However, one may speculate that synthesis of IGF I in fetal collecting duct plays a key role in organogenesis. D'Ercole et al. (6) demonstrated that IGF I is produced by explants of a variety of fetal mouse tissues including kidney. They proposed that locally produced IGF I could play a role in tissue differentiation. Consistent with this hypothesis are our recent observations (28) that IGF I gene expression in fetal rat rises approximately ninefold between embryonic days 11 and 13, a period characterized by differentiation of many organ systems including kidney (2).

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